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Comparative analysis of the composition of essential oils and supercritical carbon dioxide extracts from the berries and needles of Estonian juniper (*Juniperus communis* L.)

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Abstract

The composition of the volatile oil of the common juniper (*Juniperus communis* L.) from Estonia was analyzed by gas chromatography (GC-FID) and gas chromatography-mass spectrometry (GC-MS). The yield and composition of the oil obtained by different methods (micro-distillation and extraction, SDE, and supercritical carbon dioxide extraction, SFE) from various parts of juniper (berries, needles) were compared. The oil yield ranged from 0.7 to 2.1%. The content of α-pinene of juniper-berry essential oil was 47.9, that of juniper needleoil, 36.4%. The oil yields and composition obtained by SDE and SFE from juniper needles were similar. The oil obtained by SFE from juniper berries contained more sesquiterpenes and high boiling compounds than that obtained by SDE.

Keywords: *Juniperus communis* L.; common juniper; Cupressaceae; essential oils; SFE extracts; monoterpenes; sesquiterpenes.

1. Introduction

Common juniper *(Juniperus communis* L.) is a natural evergreen shrub or tree growing in dry uncultivated regions of Asia, Europe, North Africa and North America. The berries (*Juniperi fructus*) and needles (Juniper foliage) of juniper contain an essential oil having a characteristic aromatic flavour and bitter taste. For its diuretic and gastrointestinal properties common juniper has been known as medicinal plant for centuries. Juniper oil is a natural product which is used in the pharmaceutical and food industries and perfumery, as well as in cosmetics. Certain spirits (gin) are made by distillation from fermented juniper berries [1, 2].

The yield of the volatile oil from the berries and needles of juniper depends on the plant´s geographical location, degree of ripeness and age, as well as meteorological conditions (temperature, length of sunlight, duration of photoperiod), and other factors. The average oil yield varied from 0.5 to 2.5% (berries) and from 0.2 to 1.0% (needles) [1, 2].

In the last years a number of publications have reported the composition of the oil of the berries and needles of juniper [1–31]. Traditionally, essential oil is obtained from juniper by hydrodistillation [1–16,23–25,29,34–36].

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Its composition varies considerably, consisting mainly of monoterpenes (α-pinene, sabinene, myrcene) and sesquiterpenes (caryophyllene, muurolenes, germacrene-D and B, humulene). The major oxygenated terpenoids are terpinen-4-ol [4, 5, 9, 11, 13], citronellol [7] and terpenyl acetate [12]. The analyses of the chiral composition of the monoterpene fraction of juniper oil showed its ratio of α-pinene S to R enantiomers to greatly vary, depending on the plant´s habitat and part as well as place and season of growing [14 –16].

The composition of the oil isolated from the berries or needles of juniper by SFE was found to be highly dependant on the extraction conditions used (pressure, temperature, time of extraction, plant´s degree of grinding, choice of modifiers) [17–20]. Some less volatile compounds not found in the hydrodistilled oil were extracted under supercritical conditions. The high content of paraffins and long-chain alcohols was established in the extracts when pressure 20 MPa was used [17].

In the present study, the composition of the volatile oil obtained by SDE and SFE from the needles and berries of Estonian common juniper has been analyzed. There were quantitative differences in composition between the oils obtained by both the procedures.

2. Materials and methods

2.1. Plant material

Ripe juniper berries (J*uniperi fructus)* harvested in *2007* were purchased from a drugstore in Tallinn, Estonia (VADI GILD). Juniper needles (Juniper foliage*)* were collected at Harku near Tallinn in February 2008.

2.2. Simultaneous distillation and extraction (SDE)

The essential oil was isolated from crushed dried juniper berries and crushed fresh needles of juniper (10 g) by SDE with n-hexane (Fluka, >99.0%) as solvent (0.5 mL) by using a Marcusson-type microapparatus [37]. The SDE process was carried out during 2 h. The oil amount (%) was determined using n-tetradecane (Reachim, >99.9%) as internal standard (2 µL). The reproducibility of three parallel SDE procedures with a single juniper sample showed the variation coefficients to be below 20%.

2.3. Supercritical CO_2 extraction (SFE)

In each experiment the weight of the crushed raw material was in the range of from 0.2 to 0.4 g. SFE was performed on a Milton Roy SPA (Sample Preparation Accessory) apparatus. The extraction time was 90 minutes. The experiments were carried out at constant temperature (45 ºC, or 313 K) and pressure (120 at, or 11.8 MPa). The collecting solvent was hexane. The high purity carbon dioxide (99.5 %) from AS Eesti AGA was used. The yield of SFE extracts was determined by weighing the receiver-tube before and after the procedure.

2.4. GC-FID analysis

The SDE and SFE extracts were analyzed using a Chrom-5 chromatograph (Laboratorni Pristroje, Prague, Czech Republic) with FID on two fused silica capillary columns with two stationary phases: poly(5%diphenyl-95%dimethyl)siloxane (SPB^{TM}-5, 30 m × 0.25 mm; Supelco, Switzerland) and polar polyethylene glycol (SW-10, 30 m × 0.25 mm; Supelco, Switzerland). The film thickness of both stationary phases was 0.25 µm. The carrier gas helium with the split ratio of 1:150 and flow rate of 30–35

cm/s was used. The temperature program was from 50 - 250ºC at 2ºC/min, the injector temperature was 250ºC. For data processing a Spectra-Physics SP4100 integrator was used.
The oil components were identified by comparing their retention indices (RI) on two columns with the RI values of reference standards, our RI data and literature data [38–41]. The results obtained were confirmed by GC/MS.

2.5. GC-MS analysis

GC/MS analysis was carried out using a GCMS-QP2010 (Shimadzu, Japan) on a fused silica capillary column (30 m x 0.32 mm) with a bonded stationary phase: poly(5%-diphenyl-95%-dimethyl)siloxane) (ZB-5, Zebron). The film thickness of the stationary phase was 0.25 μm. The carrier gas helium with the split ratio of 1:17 and flow rate of 1.8 mL/min was used. The temperature program was 2 min at 60ºC and then from 60 to 280˚C at 12˚C/min, the injector temperature was 280˚C. The MS detector was operated in the EI mode of 70 eV at a scan rate of 2 cans/s with an acquisition mass range of 40–500 u.

3. Results and Discussion

The results of qualitative and quantitative analyses of the oil obtained by SDE and SFE from the berries and needles of *Juniperus communis* L. are presented in Table 1. A total of eighty seven compounds accounting for over 96% (SDE) and to 94% (SFE) of oils were identified.
The yield of oil obtained by SDE from fresh juniper needles was 0.7%, based on dry sample weigh. This compares well with the yield of oil obtained by SFE from juniper needles (0.9%). The yield of essential oil (2.1%) from dried juniper berries was almost thrice that from juniper needles. The yield of oil obtained by SFE from berries (0.9%) was similar to that obtained by SFE from juniper needles (0.9%).
Although in the essential oil of the needles and berries of *J. communis* monoterpenes predominated (49.5 and 58.0%, respectively), there were differences in composition between the compounds present. So, the juniper-needle oil contained up to 32.6% of sesquiterpenes as against 12.8% in the juniper-berry oil. The amount of oxygenated monoterpenes in the juniper-needle oil was low (2.0%), being 14.7% in the juniper-berry oil. In the juniper-needle oil α-pinene (36.4%), (E)-β-caryophyllene (8.1%), α-humulene (6.3%), β-phellandrene (6.3%), and germacrene D (4.8%) prevailed. The content of α-pinene of the juniper-berry oil was high (47.9%); the predominant minor constituents were germacrene D (3.7%), myrcene (3.4%), p-mentha-1,5-dien-8-ol (2.9%) and α-campholenal (2.4%).
Compared with literature data [4-36], the content of sesquiterpenes of the essential oil obtained from the needles of Estonian juniper was higher. The total content of β-caryophyllene, α-muurolene and germacrene D was 19.2%, while literature data report their maximum content of the oil from Italian and Spanish juniper to be 8.1 and 10.8% respectively [30, 22]. The high content of α-pinene (47.9%) of the oil from the berries of Estonian juniper compared well with that of the oil from Greek (27 - 62%) [5, 7], Polish and French (45 – 80%) [16] and Italian (52.3%) juniper [12].
Optimum SFE conditions were as follows: pressure 120 at and temperature 45ºC. 45ºC was used in order to prevent the thermal degradation of some oil components, while low pressure (120at) was applied to prevent the extraction of undesired higher molecular weight compounds (waxes and aliphatic alcohols) from the plant material [17–20, 28, 30, 32].
A comparison of the essential oils obtained by SDE and SFE from fresh juniper needles showed their compositions to be similar. The oil obtained by SFE contained 33.7% of α-pinene, 10.1% of (E)-β-caryophyllene, 6.8% of α-humulene, 5.2% of β-phellandrene, and 6.7% of germacrene D.
The oil obtained by SFE from dried juniper berries contained less monoterpenes (5.1%) and more sesquiterpenes (45.5%) and oxygenated sesquiterpenes (24.3%) than the oil obtained by SDE (58.0, 12.8, and 9.8%, respectively). In the SFE oil, germacrene D (19.0%), epi-α-bisabolol (8.9%), and (E)-β-caryophyllene (8.1%) dominated.

Table 1. The composition of the oil obtained by SDE and SFE from the berries and needles of Estonian juniper (*Juniperus communis* L.), %

Compound	RI		Berries		Needles	
	SPB-5	SW-10	SDE	SFE	SDE	SFE
n-hexanal	800	1091	0.1	nd	0.1	tr
(Z)-2-hexenal	842	1245	nd	nd	tr	tr
(E)-2-hexenal	845	1224	tr	nd	0.2	tr
2,7-dimethyloctane	900	1147	tr	0.1	tr	1.4
tricyclene	917	1010	0.2	tr	tr	nd
α-thujene	921	1020	tr	tr	tr	tr
α-pinene	930	1026	**47.9**	**3.1**	**36.4**	**33.7**
α-fenchene	937	1056	0.1	tr	tr	tr
camphene	939	1065	0.4	tr	0.1	tr
dihydrosabinene	944	1125	1.4	nd	tr	nd
sabinene	968	1121	tr	tr	0.2	0.9
β-pinene	970	1109	1.2	0.1	1.0	tr
1-octen-3-ol	978	1440	nd	nd	tr	nd
β-myrcene	988	1167	3.4	1.5	1.9	1.9
2-carene	998	1129	nd	nd	0.1	tr
α-phellandrene	1000	1163	0.5	nd	1.5	0.3
δ-3carene	1006	1146	0.2	tr	0.2	1.8
α-terpinene	1010	1178	tr	nd	tr	nd
p-cymene	1016	1272	0.6	tr	0.3	nd
limonene	1021	1200	1.2	0.2	0.7	0.7
β-phellandrene	1023	1208	tr	tr	**6.3**	**5.2**
(E)-β-ocimene	1045	1255	nd	nd	tr	nd
3-methylbutyl butanoate	1051	1269	tr	nd	tr	tr
γ-terpinene	1053	1240	tr	nd	0.1	nd
trans-4-pentenyl butanoate	1062	1346	tr	nd	tr	tr
terpinolene	1083	1283	0.5	0.2	0.7	tr
p-cymenene	1085	1426	0.4	tr	nd	nd
linalool	1100	1557	tr	0.4	tr	0.7
n-nonanal	1108	1390	nd	tr	0.1	nd
3-methylbutyl isovaleriate	1114	1374	tr	tr	tr	nd
α-campholenal	1118	1506	2.4	0.3	tr	nd
trans-pinocarveol	1130	1677	2.2	nd	tr	nd
3-methyl-2-butenyl valeriate	1132	1450	0.3	tr	tr	nd
cis-verbenol	1139	1708	1.1	0.4	nd	nd
camphene hydrate	1150	1560	0.3	nd	tr	nd
isoborneol	1155	1663	0.5	nd	tr	nd
p-mentha-1,5-dien-8-ol	1162	1665	2.9	0.2	tr	nd
terpinen-4-ol	1171	1603	1.0	0.5	0.1	tr
p-cymen-8-ol	1180	1856	0.2	nd	tr	nd

α-terpineol	1185	1700	0.8	0.3	0.2	tr
myrtenal*	1188	1631	1.0	nd	nd	nd
myrtenol*	1195	1795	0.5	nd	nd	nd
verbenone	1200	1728	1.5	0.4	tr	0.3
β-citronellol	1240	1800	tr	nd	tr	nd
3-methylbutyl hexanoate	1250	1461	0.2	0.2	nd	nd
methyl citronellate	1260	1488	tr	tr	tr	0.2
bornyl acetate	1279	1576	0.3	0.3	0.5	0.3
n-undecanone	1295	1596	tr	tr	nd	nd
myrtenyl acetate	1319	1684	tr	tr	tr	tr
α-terpinyl acetate	1343	1677	tr	nd	1.1	0.8
α-copaene	1365	1484	tr	0.1	0.4	tr
trans-myrtanyl acetate	1372	1780	nd	tr	0.1	tr
β-elemene	1382	1587	0.4	0.4	0.8	0.5
(E)-β-caryophyllene	1412	1585	**1.3**	**8.1**	**8.1**	**10.1**
γ-elemene	1420	1650	tr	tr	tr	nd
aromadendrene	1426	1600	tr	nd	tr	nd
α-humulene	1442	1656	1.2	0.1	**6.3**	**6.8**
(E)-β-farnesene	1452	1669	0.5	2.4	tr	nd
γ-muurolene	1470	1690	0.3	0.5	2.2	3.5
germacrene D	1473	1696	**3.7**	**19.0**	**4.8**	**6.7**
α-amorphene	1476	1712	0.5	0.5	0.5	tr
cadina-3,9-diene	1482	1709	0.3	0.5	0.3	nd
α-muurolene	1486	1720	0.4	2.2	1.2	0.9
eudesma-4(14),11-diene	1490	1748	0.3	tr	1.7	2.2
α-selinene	1496	1703	0.1	0.8	0.1	3.0
γ-cadinene	1500	1750	0.8	2.9	0.5	0.9
β-bisabolene*	1507	1732	0.7	1.4	0.1	nd
δ-cadinene	1515	1752	1.1	0.5	2.2	1.0
α-farnesene	1528	1740	tr	0.9	tr	nd
β-elemol	1540	2078	tr	nd	tr	nd
germacrene B	1542	1815	1.2	5.2	3.4	3.6
spathulenol	1560	2118	1.2	0.4	0.6	0.4
caryophyllene oxide	1562	1965	0.6	3.0	1.5	1.4
germacren D-4-ol	1567	2045	1.2	0.7	0.5	tr
epiglobulol	1580	2112	0.3	nd	tr	nd
1,2-epoxy-1,5,8,8-tetramethyl-undeca-5,9-diene	1596	2021	1.2	5.6	0.7	0.7
humulene epoxide	1606	2049	0.1	0.7	0.1	0.7
cis-ledol	1617	2100	0.5	0.5	tr	1.9
δ-cadinol	1632	2165	0.8	0.2	0.3	tr
caryophyllenol*	1634	2358	nd	nd	3.4	tr
epi-α-cadinol*	1637	2182	0.2	2.1	1.1	tr
T-muurolol	1641	2195	nd	1.0	0.3	tr

α-cadinol	1645	2227	1.7	0.5	1.1	tr
(Z,Z)-α-cadinol	1679	2286	0.2	0.3	0.3	tr
epi-α-bisabolol	1682	2219	1.7	8.9	tr	nd
(Z,E)-α-farnesol	1700	2316	0.1	0.4	0.7	1.9
(E,E)-α-farnesol	1724	2352	tr	1.1	nd	nd
Compound groups:						
Monoterpenoic hydrocarbons			58.0	5.1	49.5	44.5
Oxygenated monoterpenes			14.7	2.8	2.0	2.3
Sesquiterpenoic hydrocarbons			12.8	45.5	32.6	39.2
Oxygenated sesquiterpenes			9.8	24.3	10.6	7.0
Other compounds			0.6	0.3	0.4	1.4
Total, %			95.4	78.0	95.1	94.4
Oil yield, %			2.1	0.9	0.7	0.9

tr – traces (<0.05%), nd – not detected, * - identified by GC-FID.

4. Conclusions

This work showed the composition of the oil obtained by SFE with CO_2 at moderate conditions from fresh common juniper needles to be similar to that obtained by SDE. The oil obtained by SFE from dried juniper berries contained more sesquiterpenes and high boiling compounds than that obtained by SDE.

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